On the Histological Structure of the Retina of the Lateral Eyes of Sphenodon punctatus, with Special Reference to the Sense-cells.

By

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With Plates 27—29.

I. Introduction.

The following work on the structure of the retina in the lateral eyes of Sphenodon punctatus has been done in the Zoological Department at King's College (University of London), at the suggestion and with the help of Professor Dendy. I have to thank him both for his help and advice throughout, and for the use of his valuable collection of Sphenodon material. I have not only had a considerable number of eyes at my disposal, but I found them in an excellent state of preservation for histological work. I should like here to thank, also, Mr. R. W. H. Row, B.Sc., for his kindness in taking photographs. The investigations are based entirely on microscopical preparations made from this already preserved material. No fresh material has been available, and for this reason I have found it advisable to confine the detailed work for the most part to the structure of the visual cells, dealing, as regards the remainder of the retina, only with those parts which are visible without special treatment for nerve-fibres.

The structure of the retina of various vertebrates has engaged the attention of many workers, especially during the last eight or ten years. The greater part of the work (apart from that on the human eye) has been done on amphibians, while a certain number of papers have also been published dealing with birds and fishes. So far as I have been able to ascertain very few investigations have been made on reptilia of any kind.

As the literature of the visual cells in vertebrates generally has been fully discussed by Howard (**19**) as recently as 1908, it is unnecessary to enter into historical details in this paper.

A paper dealing with the lateral eyes of Sphenodon was published by Osawa (**23**) in 1898. So far as the description of the structure of the retina goes, I find that my results do not agree with his at all. He begins by stating that, unlike other Reptilia, Sphenodon possesses in its retina both rods and cones, and that oil-globules occur in both. His figures are very unconvincing and wanting in detail. So far as I can make out from them, and from the text, he regards as cones only the large flask-shaped cells which are generally described as the "near cones" of the "double cones." He says he must leave undecided the question whether or not these are to be regarded as "Hauptzapfen" (i. e. near cones). He figures an oil-globule in the "Hauptzapfen," which certainly does not exist in the near cones, and he calls the paraboloid the ellipsoid. Now it is generally admitted, by those who have worked at the subject, that the cones of certain groups of vertebrates possess oil-globules, but that the rods never do. Osawa, however, describes as rods sense-cells which possess oil-globules, but are of a somewhat different shape and size from those which he figures as cones. These so-called rods (Stäbchen) are what I shall speak of later as ordinary single cones. He also finds some intermediate forms which he calls "doubtful cones or rods," and these are probably the small forms which I suppose to be small single cones. He states that he finds cones grouped in

pairs (presumably double cones) in isolation preparations, but very seldom joined together to the foot. All these sense-cells resemble so closely the generally accepted types of cones in other forms, that it seems unnecessary to regard Sphenodon as an exception to the general rule in Reptilia that cones only are present without rods.

Further, Osawa states that he could not find any distinct "area centralis" (i. e. macula lutea). I find, on the other hand, that the macula lutea is well developed and possesses a perfectly distinct fovea centralis as shown in figs. 1 and 2. Kallius (**20**) has since, in answer to Osawa, described and figured a well-formed "area centralis" in Sphenodon. He mentions that rods are present in the central fovea very closely packed together, but the oil-globules show these to be really cones. His photograph of the area centralis quite closely resembles the appearances I have seen. Osawa (**24**), in reply, states that he has seen the "area centralis" mentioned by Kallius, but in his subsequent account he still appears to think it coincident with the blind spot. That this is not the case will be seen in my fig. 1. Kallius' material does not appear to have been in a very good state of preservation, and he gives no histological details as to the structure of the retina.

II. Methods.

When this work was begun all the available paired eyes of the specimens used by Professor Dendy for his memoir (**8**) on "The Pineal Organs and Adjacent Parts of the Brain in the Tuatara (Sphenodon punctatus)" had, as I have above stated, been already fixed and preserved. The material may be classified as follows.

(A) Flemming Material.—The eyes of Sphenodon V[1]

[1] The numbers are those given by Professor Dendy in the memoir referred to, p. 231, but the lateral eyes were in all cases removed and preserved quite separately and sometimes by different methods from those employed by Professor Dendy in his own investigations.

had been preserved in Flemming's solution, and two of these were cut. They gave very good results so far as the shape of the visual cells is concerned. There was apparently little or no shrinkage, but I find that sections of material fixed in this way do not stain readily, owing, of course, to the osmic acid employed. The most useful results with this material were obtained by staining in bulk with borax carmine, and on the slide with brazilin. There is very little differentiation in colour by this method, the sections being of a brownish tint for the most part; but the nuclei throughout are well marked, their chromatin showing most distinctly, and the paraboloids of the sense-cells staining with a slight pink tinge in contrast with the rest of the sections. The oil-globules are, of course, quite black, owing to the osmic acid, and the outer segments show good detailed structure and are not much broken, though somewhat shrivelled.

Iron-hæmatoxylin gives fair results, the nuclei being well stained and of a bluish tinge, but the different parts of the sense-cells are not well differentiated.

On some of this material, which had not been previously stained in bulk, I tried a ready-made "Weigert Pal" stain obtained from Grubler. It was not possible to subject the already hardened and preserved material to all the preliminary processes which form a part of this method, and the sections obtained, though they were useful, were practically unstained, and resembled those cut and mounted without any staining on the slide, being of a yellowish tinge with black oil-globules and outer segments. I made use of them, however, because the nerve-fibrils of the various cells, though unstained, were unusually distinct.

(B) Zenker Material.—The paired eyes of Sphenodon III, which had been fixed in Zenker's fluid, were excellent for the general histology and cytology of the sense-cells. The eyes were stained in bulk with borax carmine and various other stains were used on the slide.

Picro-indigo-carmine gave excellent results, differentiating the various layers and cells in shades of green and red. The

finer details of the cones were clearly expressed, the paraboloid, the ellipsoid and the outer segment all staining in different tints as will be described in due course.

Picro-nigrosine, though rather erratic in its staining effects, was in places excellent, and some of my best sections were prepared in this way, the details showing well in red and grey colours.

The oil-globules remained unstained in all these preparations.

(c) Aceto-bichromate Material.—The eyes of Sphenodon VI had been fixed in acetic bichromate of potash and proved most satisfactory. The layers of the retina separated less than in the case of material fixed in the other fluids. The sections stained readily and their histology was excellent.

My most successful preparations were those stained in bulk with borax carmine and on the slides afterwards with picro-indigo-carmine, when the general appearance was as in those fixed in Zenker's fluid.

The oil-globules remained unstained after acetic bichromate as after Zenker.

I should like to take this opportunity of expressing my indebtedness to Mr. Charles Biddolph, Prof. Dendy's laboratory assistant, for his skilful assistance in preparing the sections.

III. Descriptive and Critical Account of the Retina.

A. General Account.

The retina of the preserved eye, as in other animals, very readily separates into two layers, the pigment epithelium remaining attached to the choroid and the inner portion shrinking away from it.

On cutting the eyeball at right angles to the optic axis and looking at the inner surface of the posterior half, a small pit can be distinctly seen lying near the optic axis and at a distance of about 3·5 mm. from the point of entrance of the

optic nerve. The pit is seen in sections to be a well-developed central fovea (136 μ deep at its centre), with a rather less well-marked though still distinct macula lutea surrounding it. The optic nerve has the usual position and relations, and both it and the yellow spot are clearly shown in fig. 1.

An examination of vertical sections shows the usual eight layers of the vertebrate retina all well developed and with the normal characteristics. The retina is thickest around the central fovea, in the region of the macula lutea, and from there gradually decreases in thickness in all directions towards the ora serrata, where it changes its histological character, and the pars ciliaris retinæ, consisting of columnar cells and a layer of pigment, begins (fig. 13).

The thickness of the retina is as follows:

(1) In the fovea centralis, 204 μ; (2) in the macula lutea, 306 μ; (3) half way between the fovea and the ora serrata, 237 μ; (4) in the pars ciliaris, 17 μ.

As usual in the lower vertebrates no blood-vessels are present in any part of the retina. Quain (**32**) states that the Chelonia form an exception to this rule—an interesting fact in view of the supposed relationship between this group and the Rhynchocephala.

We may now consider the histological structure of the different layers of the retina separately.

(1) Nerve-fibre Layer (numbered *1* in figs. 2, 4 and 12).

Nerve fibres, of course, branch out from the optic nerve (fig. 1, *o.n.*) in all directions from its point of entrance, forming a very definite layer on the inner surface of the retina (figs. 4 and 12, *1*). This becomes thinner and thinner towards the front of the eye, disappearing altogether at the ora serrata (fig. 13, *or.s.*). The nerve-fibre layer is also absent from the central fovea (fig. 2, *f.c.*) and is extremely thin over the macula lutea, where most of the other layers thicken around the central fovea (fig. 2, *1*). One very definite band of nerve-fibres may be seen with the naked eye on the inner surface of the wall of the eyeball, commencing

at the blind spot. It ramifies over the retina, gradually diminishes in size, and disappears as it gives off its branches. In sections cut through and parallel with the optic axis this main trunk is seen to be from two to four times the thickness of the nerve-fibre layer over the rest of the retina. The aggregation of the nerve-fibres in bundles is very distinct throughout the retina (fig. 12, *n.f.b.*). In all parts of the retina, so far as I could ascertain, the nerve-fibres are non-medullated.

(2) Ganglionic Layer (numbered *2* in figs. 2, 4, 12).

Though forming a definite and fairly regular layer, the actual ganglion cells are of various sizes and shapes, and some of their nuclei stain much more deeply than others. In a few cases only were the branches of the cells seen passing into the inner molecular layer, and the nerve-fibre passing out from the ganglion cell was also seen only occasionally. This was due again to the method of staining, which was adapted more to the cytological investigation of the visual cells, and was not suitable for the nerve-fibres.

The distribution of the ganglion cells over the retina is easily determined by the position of the well-stained nuclei. They are scattered very irregularly round the entrance of the optic nerve in a single layer only. In all directions round this irregular part they are placed side by side together, but still in a single layer (figs. 4 and 12, *n.g.c.*). This becomes irregular again towards the ora serrata (fig. 13, *n.g.c.*), and then ceases altogether in the pars ciliaris (fig. 13, *p.c.r.*). A special arrangement of the ganglion cells is found over the macula lutea where the layer becomes double (fig. 2, *m.l. 2*). In one or two sections there appear to be even four layers in this neighbourhood, but the latter appearance is, I think, due to the sections being somewhat oblique. Towards the centre of the yellow spot the double layer becomes single (fig. 2, *2*), and in the central fovea itself (fig. 2, *f.c.*) the ganglion cells are very much scattered, only

four or five showing in each section at long distances from one another.

(3) Inner Molecular Layer (numbered *3* in figs. 2, 4, 11, 12).

This is composed, as usual, of masses of fibrillæ, the transverse sections of which give the characteristic punctate appearance seen in sections (figs. 11 and 12, *3*). This layer is present throughout the retina proper, decreasing gradually in thickness towards the ora serrata, where it ceases. In the fovea centralis (fig. 2, *f.c.*) it diminishes to about two thirds of the thickness which it has over the general part of the retina.

(4) Inner Nuclear Layer (numbered *4* in figs. 2, 4, 11).

This layer, in thickness usually about the same as the inner molecular layer, contains numerous deeply staining nuclei of three kinds, easily distinguishable from one another by their size, shape, and staining properties. All three kinds are commonly seen in this layer (Quain, **32**).

(A) Spherical nuclei which stain very deeply (figs. 4, 11, *n.b.*). These are present in by far the greatest numbers, and are the nuclei of bipolar nerve-cells. In a few cases their attached fibres can be seen.

(B) Much larger spherical nuclei which stain less deeply and belong to multipolar nerve-cells (figs. 4, 11, *n.m.*).

(C) Oval nuclei about the size of (A) (figs. 4, 11, *n.m.f.*). They take the stain very deeply, and are always placed with their length at right angles to the surface of the retina. These are the nuclei of the supporting Müller's fibres (*m.f.*) in which they lie.

Over most of the retina the inner nuclear layer is from 36–40 μ in thickness, gradually decreasing towards the ora serrata. At the macula lutea (figs. 1 and 2) there is an enormous increase in the number of cells present, and the thickness of the layer becomes rather more than doubled. It

decreases again at the central fovea to about 30 μ, and here there is a proportionately larger number of multipolar nerve-cells.

(5) Outer Molecular Layer (numbered *5* in figs. 2 and 4).

This is similar in appearance to the inner molecular layer, being made up of a network of fibrils. In this case, however, the fibrils come from the visual cells, and interlace with processes from the bipolar and multipolar nerve-cells of the inner nuclear layer. The outer molecular layer, though very definite, is much less thick than the inner molecular (measuring about 15 μ), and unlike the latter does not vary appreciably in thickness over the whole retina up to the ora serrata, including the macula lutea and fovea centralis (fig. 2).

(6) Outer Nuclear Layer (numbered *6* in figs. 2, 4).

The cells here are much more definitely arranged than those of the inner nuclear layer. The whole thickness is from one half to one third that of the latter, and only two kinds of nuclei are present. The chief of these are those of the visual cells (figs. 4, *nuc.*), which form a more or less regular single layer towards the outer limit. They are somewhat larger than any of the nuclei of the inner nuclear layer, distinctly oval in shape, and in most of my preparations have very distinct chromatin granules, and sometimes show a definite nucleolus. In many cases (fig. 7, *c.n.*) the cytoplasm is seen to be continued from the inner segment of the cone as a thin investment over the nucleus, and from this a distinct fibril (fig. 7, *c.c.f.*) passes through the thickness of the whole layer to ramify in the outer molecular layer (fig. 7, *r.c.f.*). The shape and position of these nuclei vary somewhat according to the size of the cones, those (fig. 8, *nuc.*^{2}) of the extremely small cones, with which we shall deal presently, being more pointed at their outer ends and further away from the membrana limitans externa than those of the ordinary single and double cones, which almost or quite touch the

external limiting membrane, so that their outer ends become somewhat flattened (fig. 7, *nuc.*[1, 3, 4]). In tangential sections the nuclei of the cones are seen to be connected by a syncytial network of protoplasm (fig. 10, *syn*) formed by the extension of the cytoplasmic investment of each. Bernard (**3**, **4**, **5**), regards the retina as composed not of cells, but of a syncytium in which the nuclei are arranged in layers. The cytoplasm connecting the nuclei of the cones in Sphenodon certainly forms a network, but at the same time the connection of the nuclei with the inner segments is so definite that I must agree with most writers that the sensory epithelium is a definite layer of cells whose nuclei are situated in the outer nuclear layer. Each member of one of the so-called double or twin cones has a nucleus which does not differ perceptibly in shape or size from those of the ordinary single cones (figs. 7, *nuc.*[3, 4]).

The second kind of nucleus present in the outer nuclear layer attracts attention at once. They are rounded and arranged in a more or less definite single layer close to the junction with the outer molecular layer (figs. 4 and 7, *n.d.b.*). Each is surrounded by a definite space, due doubtless to shrinkage, and resembles in appearance the nucleus of one of the bipolar cells of the inner nuclear layer (figs. 4, 11, *n.b.*). According to various authors these nuclei are to be regarded as belonging to bipolar nerve-cells which have migrated from the inner to the outer nuclear layer. Gaupp (**30**) figures them in the frog's retina, and calls them "Versprengte Bipolare." I could find no trace of fibrils coming from them, even in the preparations which showed fibrils in other parts, but owing to the nature of my material this proves nothing as to the existence or non-existence of such fibrils.

The outer nuclear layer is bounded on its outer aspect by the membrana limitans externa (figs. 2, 4, 5, 8, etc., *m.l.e.*), appearing in vertical sections as a very definite, darkly staining line, and formed according to most authors by the outer parts of the Müller's fibres.

(7) Layer of Cones (numbered *7* in figs. 2 and 4).

There are no rods present in Sphenodon, in which respect this animal agrees with lizards, snakes, and tortoises. Indeed the absence of rods is common to all reptiles, as stated by Gamgee (**29**). The cones are of several distinct kinds, both single and double being present in large numbers, and in some cases much smaller ones (figs. 8, *S.S.C.*) occurring between the ordinary single ones (fig. 8, *O.S.C.*). This layer is, of course, made up only of those parts of the visual cells which project beyond the membrana limitans externa, the portions which contain the nuclei lying in the outer nuclear layer, as already stated. It is continuous over the whole of the retina proper (except, of course, the blind spot). The cones measure in length from 37 to 50 μ, the different sizes being intermingled with one another over the greater part of the retina as shown in fig. 5. Towards the ora serrata they become shorter and thicker, and gradually diminish in number until they completely disappear in the pars ciliaris. Over the macula lutea they are somewhat longer and correspondingly thinner than in any other part, and more closely packed together. As might be expected the most densely packed cones are the longest and narrowest, so that at the central fovea there are fifteen cones to every six in the main part of the retina, the corresponding number in the macula lutea around the central fovea being eight.

Over the macula lutea the pigment epithelial layer of the retina, usually so easily separated, remains attached to the cones, as clearly shown in figs. 1 and 2.

(8) Layer of Pigment Epithelium (numbered *8* in figs. 2 and 4).

Each cell of this layer consists of an outer portion which abuts against the choroid, from which it is separated by a very distinct membrane, and an inner portion broken up into long processes (fig. 4, *p.p.*) which pass inwards between the outer segments of the cones. The outer portions fit closely

together as a pavement epithelium, the boundaries between them being pentagonal and hexagonal and very distinct (fig. 14, *c.w.*). This part of each cell contains the nucleus (figs. 4 and 14, *n.p.e.c.*) with a very distinct nucleolus (fig. 14, *nucl.*), and the surrounding cytoplasm is free from pigment, but exhibits a curious "curdled" appearance as if broken up into irregular blocks (figs. 14, *bl.p.*). The inner portions are made up of long and slender threads, each containing a great number of pigment granules. The latter often remain attached to the outer segments of the cones when the pigment layer has been torn away (fig. 5, *p.g.*).

(9) The Supporting Structures of the Retina (figs. 4, 5, 6, 7, 8, 11, 12, 13).

Müller's fibres support the various layers of the retina and pass from the innermost or nerve-fibre layer to the bases of the cones. Inside the nerve-fibre layer they spread out into trumpet-shaped bases (fig. 12, *m.f.b.*), which unite to form a definite layer over the inner surface of the retina. This is the membrana limitans interna (figs. 2, 4, 12, etc., *m.l.i.*). The fibres (fig. 12, etc., *m.f.*) pass up between the ganglion cells and through the inner and outer molecular and nuclear layers, and finally spread out again to form a membrane (fig. 4, etc., *m.l.e.*), comparable with the internal limiting membrane, which covers the outer surface of the outer nuclear layer, and through which the cones project. Small projections (figs. 5, 6, *p.m.f.*) extend between the cones beyond the external limiting membrane, and these probably serve to support the bases of the cones. The nuclei of Müller's fibres are situated in the inner nuclear layer (fig. 11, *n.m.f.*). They are large, oval in shape, and stain quite readily and darkly.

Verhoeff (28) contends that the external limiting membrane is not formed from Müller's fibres, but is a product of the embryonic cells which became converted into the rods and cones. His work was done on the human eye, and I am not in a position to state whether the same is the case in

Sphenodon, in which, however, I have found no reason to doubt the correctness of the older interpretation of the membrana limitans externa as being formed from the Müller's fibres.

B. Detailed Account of the Sense-Cells.

Sphenodon appears to form no exception to the rule that cones only are present, to the exclusion of rods, in the reptilian retina. It is generally admitted that oil-globules do not occur in rods, but they are present in nearly all the visual cells in Sphenodon. The only ones in which there are no oil-globules are the larger members of the double or twin cones—i.e. the so-called near cones—and these have other distinctive characteristics which mark them out as cones, viz. their association with the smaller members to form the well known pairs, and their very characteristic shape. There are three very definite kinds of cones in Sphenodon, viz. the smaller and larger single cones and the double cones. The two members of the latter, though always associated, have so many differences that they may be conveniently considered separately. I shall classify the cones, then, as follows:

(1) Ordinary single cones; (2) small single cones; (3) double cones—(a) near cones, (b) far cones.

In the fovea centralis only cones of the first type are present, densely packed together, and somewhat longer and more slender than in other parts of the retina. In the macula lutea, outside the fovea, a few double cones are present also, and the single ones are less closely packed, the number in a given space being only about half what it is in the central fovea. Over the main part of the retina (fig. 5), many more double cones are present, though they are still less numerous than the single ones, there being only about one double cone to every two ordinary single ones. Ordinary single cones only are present towards the ora serrata, where they gradually become shorter and thicker and finally disappear altogether.

(1) The Ordinary Single Cones (figs. 3–9).

These are most numerous. The outer segments (figs. 4, 5, *o.s.*[1]) are conical in shape and in most of my preparations are more or less broken up into what appear to be flat, plate-like discs (fig. 5, *d.o.s.*). In quite a number of cases, however, part of the outer segment has the appearance of a closely wound spiral of two parallel threads (fig. 6, [b], *s.o.s.*). This is only the case with the inner half of the outer segment, i. e. the half next the oil-globule, and in these cases the outer half still appears as if broken up into discs (fig. 6 [b], *d.o.s.*). It is in preparations of the eye fixed in Flemming's solution (fig. 6) that the spiral, black in colour owing to the osmic acid in the solution, is to be seen, and it seems to be due to shrinkage. Acetic bichromate material does not show it, only the discs being visible here in all parts of the outer segments (fig. 5, *d.o.s.*). They stain very slightly after this fixation, becoming pale yellowish in colour after picro-nigrosine or picro-indigo-carmine.

A great deal of controversy has taken place over the structure of the outer segments of the visual cells of vertebrates. All workers are agreed in finding them very unstable, and no doubt the varying results obtained are due to the different appearances assumed after treatment of the various retinas with different fixing fluids and stains.

Two main views are held as to the structure of the outer segments of cones. Many preparations have been obtained which show them broken up into cross discs (as in fig. 5, *d.o.s.*). This is given in the various text-books (Gamgee **29**, Halliburton **31**, Quain **32**). Heinemann (**14**) describes an annular breaking up into transverse plates in the outer segments of the cones in Chelonians, and Dogiel (**11**) states that the outer segments are ringed in some cases in Ganoids. The appearance seen in some of my preparations (fig. 6 [b], *s.o.s.*), gives rise to another view, viz. that the outer segments are spiral. Hesse (**15**) gives an interesting historical resumé

of this theory. The spiral has been regarded by some as continuous with longitudinal fibrils in the inner segment of the visual cell (Ritte, 1891), by others as distinct from the inner segments and having no connection with it (Krause, 1895). Hesse himself writes of a double spiral twisting in bony fishes, amphibians and reptiles, but could not trace the end of it. Howard (**18**) describes a well marked axial core in the outer segment of each rod in the retina of various vertebrates. These rods in the fresh state showed transverse banding. In a later paper (**19**) he figures a spiral appearance of the outer segments of certain cones in Necturus, and regards it as possibly due to the irregular separation of the transverse discs by vacuoles.

Bernard (**2**, **3**, **4**, **5**) holds an entirely different view as to the structure of these outer segments. They are, according to him, irregular masses of protoplasm, squeezed out from the inner segments of the rod or cone-cells, and passing between the processes of the pigment epithelium. He only gets these results in certain preparations, and his figures suggest that they are due to imperfect fixation of his material.

Between the outer and inner segments of the ordinary single cones in Sphenodon is a large globule of a fatty or oily nature which turns black with osmic acid, i.e. in Flemming preserved material (figs. 4, 6, 7, 8, *o.g.*). Such oil-globules are well known to be characteristic of the cone-cells of birds, reptiles and amphibians (Schäfer, **33**). After fixing with Zenker's fluid or acetic bichromate the oil-globule remains unstained with all the stains I have used (fig. 5, *o.g.*).

The inner segment of the cone is somewhat cylindrical in shape, and contains the ellipsoid and the paraboloid. The former lies next to the oil-globule, and the latter nearer the membrane limitans externa. The ellipsoid differs much in detailed appearance according to the method of preparation. In material fixed in acetic bichromate and stained with picro-nigrosine it has a slightly granular appearance, and is possibly vacuolated (fig. 5, *ell.*[1]). In osmic acid (Flemming) material it has a denser and much more uniform appearance

(figs. 4, 6, 7, *ell.*[1]). It is less definite in shape than the paraboloid, and in some preparations both the oil globule and paraboloid press in on each side causing the ellipsoid to become biconcave (fig. 5, *ell.*[1]).

In transverse section the ellipsoids are somewhat pentagonal in shape, owing to the pressure of the cones against one another. In one preparation (fig. 9, *ell.*[1]), fixed with acetic bichromate and stained rather deeply with picro-indigo-carmine, the ellipsoids of the ordinary single cones are stained dark green in colour and show no vacuolation.

The paraboloid (figs. 4, 5, 6, *par.*[1]) is very definitely oval in shape and is bounded by a thick wall (figs. 5 and 6, *w.p.*), which stains distinctly. In the picro-nigrosine preparations (fig. 5) it is purple in colour. The contents of the paraboloid stain much less readily, though in all cases there appears to be a slight coagulation which can sometimes be resolved into a network of fibrils (figs. 5, 6 and 7, *f.n.*). A definite basal part of the cone (figs. 5 and 6, *b.c.*) is seen between the paraboloid and the external limiting membrane. It is somewhat granular in appearance in the picro-nigrosine sections and simply forms the ground-work or supporting structure in which the paraboloid is embedded, this basal cytoplasm being continuous with that passing over the nucleus (fig. 7, *c.n.*) into the cone-cell fibril. When the cone is cut through its long axis it is seen that the paraboloid almost, or quite, touches the nucleus (fig. 7).

The size of the ordinary single cones varies somewhat in different situations. Over the greater part of the retina they measure about 40 μ in length. In the fovea centralis, where only cones of this type are present, they are, as already mentioned, very densely packed and somewhat longer and more slender, those of the macula lutea outside the central fovea being intermediate in size and form.

(2) The Small Single Cones (fig. 8).

Quite small cones (fig. 8, *S.S.C.*) are to be seen here and there among the ordinary single ones. These, in spite of

their small size, have much the same structure as the ones described above. Their nuclei (fig. 8, *nuc.*[2]), however, as mentioned before, are quite distinct in shape and position from those of all the other cones. Fig. 8 shows two of these small cones. The larger one (*c*) has a well formed oil globule, ellipsoid, and paraboloid. The smaller (*d*) shows the ellipsoid, but neither oil globule nor paraboloid, which have probably been missed owing to the plane of section.[1] These small cones may possibly be young ones which are thrusting themselves up between the old ones, but there is no evidence to show how new sense-cells really originate. In this connection I may point out that, according to Bernard (**2**), the cones in other forms are merely stages in the development of the rods.

(3) The Double Cones (figs. 3, 5, 7, 9).

The so-called double or twin cones are paired structures, each pair consisting of a large "near cone" and a small "far cone," always found in conjunction with one another. Both "near" and "far" cones differ considerably in size, shape and minute structure, not only from the ordinary and small single cones but also from one another. Double cones are much less numerous than the ordinary single cones, there being about half the number over a given area.

In a paper published in 1900, Eigenmann and Shafer (**13**) point out that in many fishes distinct patterns are found by the arrangement of the double and single cones. Certain arrangements of these cones are apparent in the retina of Sphenodon, notably horse-shoes of single cones arranged round a double one (fig. 9, *j*). This pattern is present in many parts of the retina, but so many other apparently irregular arrangements occur also that one cannot say that the cones are arranged on any definite mosaic in this case.

(*a*) The near cone (figs. 5, 7, and 8, *N.C.*) is very much larger than the ordinary single cone, the difference in size

[1] The outer segment also is not shown in either case.

concerning especially the inner segment, for the outer segments of all the cones (fig. 5, *o.s.*[1,3,4]) are practically alike. The entire cone is flask-shaped with a strongly swollen inner segment, both paraboloid and ellipsoid being of relatively enormous size. No oil globule is present. The ellipsoid is less definite in shape even than that of the ordinary single cone; it is very strongly vacuolated in appearance, and fine dots are present (fig. 5, *ell.*[3]), which are in all probability the points where the walls of the vacuoles meet one another. Towards the distal end of the ellipsoid the vacuoles are much larger, though they still appear to be arranged irregularly. A more definite arrangement of vacuoles is seen in transverse section. On the side of the ellipsoid nearest to the stalk of the far cone they sometimes appear to be arranged in a single row (fig. 9, *e.*). If the section happens to pass through the distal portion of the ellipsoid they may appear as shown in fig. 9 (*f*).

The paraboloid measures 15·5 μ in length, nearly twice the length of that of the ordinary single cones. It has a thick wall like that of the paraboloids of the single cones, except at the distal end, where the wall at first sight appears to be missing, so that the ellipsoid seems to rest as upon the open mouth of a cup (fig. 5, *w.p.t.*); but there is probably a thin wall to the paraboloid even here. The contents of the paraboloid stain in various ways. With picro-nigrosine after fixation with acetic bichromate (fig. 5), a slightly staining coagulum becomes visible, purplish in colour. The Flemming fixed material, unstained or with the Weigert Pal stain,[1] shows the contents more definitely coagulated, forming a yellowish network in which the nodes show as dark spots (fig. 7, *f.n.*). In the same material, stained on the slide with brazilin, after being stained in bulk with borax carmine, the paraboloids of these cones became a distinct pink colour, while those of the others remained practically colourless.

The connection of the inner segment of these cones with the nucleus takes place as in the case of the ordinary single

[1] See "Methods," p. 308, for explanation of this preparation.

cones, the nucleus (fig. 7, *nuc.*[3]) being oval and having its outer extremity more or less flattened against the paraboloid itself.

(*b*) The far cone (figs. 5, 7, *F.C.*) is modified in several ways. The outer segment (fig. 5, *o.s.*[4]) is of the same structure as that of the various other cones. An oil globule is present as in the ordinary single cone, and the ellipsoid (figs. 5, 7, 9, *ell.*[4]), in detailed structure, also resembles that of the single cones rather than that of the more modified near cone. No paraboloid is present, its place being taken by a definite stalk (figs. 5, 7, 9, *St. F.C.*), connecting the distal portion of the cone with its base in the outer nuclear layer. In longitudinal section (vertical section of the retina) this stalk appears as a fine thread, but when seen in transverse section the arrangement is much better understood. The stalk (fig. 9, *St. F.C.*) is then seen to be flattened against the near cone, and to be spread over one side of the latter like a sheath, as shown in fig. 9 (*g*). In many of these flattened stalks definite fibrils can be seen cut in transverse section, as shown in fig. 9 (*h*), and these no doubt support the stalk. The far cones have each a definite nucleus, as have the near ones, and thus the double cones have each two distinct nuclei. These were distinguishable in most cases and I have no doubt that two are always present. Howard (**19**, p. 619) also distinguishes two nuclei in Necturus in a great many cases, and does not agree with Schultze, who states that one nucleus is present in the double cone.

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EXPLANATION OF PLATES 27—29,

Illustrating Freda Bage's paper "On the Histological Structure of the Retina of the Lateral Eyes of Sphenodon punctatus, with Special Reference to the Sense-Cells."

Reference Letters.

1. Nerve-fibre layer of retina. *2.* Ganglion-cell layer of retina. *3.* Inner molecular layer of retina. *4.* Inner nuclear layer of retina. *5.* Outer molecular layer of retina. *6.* Outer nuclear layer of retina. *7.* Layer of sense-cells. *8.* Layer of pigment epithelium. *D.C.* Double

cone. *F.C.* Far cone of double cone. *N.C.* Near cone of double cone. *O.S.C.* Ordinary single cone. *S.S.C.* Small single cone. *St. F. C.* Stalk of far cone. *b.c.* Base of cone. *bl. p.* Protoplasm arranged in blocks. *b. s.* Blind spot. *b. v.* Blood-vessels. *c. c.f.* Fibril from cone-cell. *ch.* Choroid. *chr.* Chromatin granules. *c. m.* Multipolar nerve-cell. *c. n.* Cytoplasm of cone passing over nucleus. *c. w.* Cell-wall. *d. o. s.* Discs into which outer segment is broken up. *ell.* Ellipsoid of cone. *ell.*[1] Ellipsoid of ordinary single cone. *ell.*[2] Ellipsoid of small single cone. *ell.*[3] Ellipsoid of near cone of double cone. *ell.*[4] Ellipsoid of far cone of double cone. *f. c.* Fovea centralis. *f. n.* Network of fibrils in paraboloid. *i. s.* Inner segment of cone. *m. f.* Müller's fibre. *m. f. b.* Trumpet-shaped base of Müller's fibre. *m. l.* Macula lutea. *m. l.* 2. Double layer of ganglion cells at the macula lutea. *m. l. e.* Membrana limitans externa. *m. l. i.* Membrana limitans interna. *n. b.* Nucleus of bipolar nerve-cell. *n. c. c.* Nucleus of columnar cells of pars ciliaris retinæ. *n. d. b.* Nucleus of displaced bipolar nerve-cell. *n. f. b.* Bundle of nerve-fibres. *n. g. c.* Nucleus of ganglion-cell. *n. m.* Nucleus of multipolar nerve-cell. *n. m. f.* Nucleus of Müller's fibre. *n. p. e. c.* Nucleus of pigment epithelial cell. *nuc.* Nucleus of cone cell. *nuc.*[1] Nucleus of ordinary single cone. *nuc.*[2] Nucleus of small single cone. *nuc.*[3] Nucleus of near cone of double cone. *nuc.*[4] Nucleus of far cone of double cone. *nucl.* Nucleolus. *o. g.* Oil-globule of cone. *o. n.* Optic nerve. *or. s.* Ora serrata. *o. s.* Outer segment of cone. *o. s.*[1] Outer segment of ordinary single cone. *o. s.*[3] Outer segment of near cone of double cone. *o. s.*[4] Outer segment of far cone of double cone. *par.* Paraboloid of cone. *par.*[1] Paraboloid of ordinary single cone. *par.*[2] Paraboloid of small single cone. *par.*[3] Paraboloid of near cone of double cone. *p. c. r.* Pars ciliaris retinæ. *p. e. c.* Pigment epithelial cells. *pg.* Pigment. *p. m. f.* Part of Müller's fibre which projects beyond the membrana limitans externa. *p. p.* Pigment processes from pigment epithelial cells. *r. c. f.* Ramifications of cone fibril in outer molecular layer. *ret.* Retina. *scl.* Sclerotic. *s. o. s.* Spiral part of outer segment of cone. *syn.* Syncitial network connecting the bases of the nuclei of the cone-cells. *w. p.* Wall of paraboloid. *w. p. t.* Thin part of wall of paraboloid in near cone of double cone.

PLATE 27.

[All the figures are from photographs of preparations of the retina of Sphenodon punctatus kindly taken for me by Professor Dendy and Mr. R. W. H. Row.

Fig. 1.—Wall of the eyeball cut through the entrance of the optic nerve and the macula lutea. The sclerotic and choroid layers are

shown and the several layers of the retina. The pigment is separated from the other layers except at the macula lutea. The central fovea is cut a little to one side. From an eye fixed by acetic bichromate and stained with Ehrlich's hæmatoxylin. × 20.

Fig. 2.—Vertical section through a portion of the choroid and of the retina, showing the arrangement of the various layers of the retina at the fovea centralis and the surrounding macula lutea. From an eye fixed with acetic bichromate and stained in bulk with borax carmine, and on the slide with picro-indigo-carmine. × 160.

Fig 3.—Tangential section through part of the retina, showing the pigment epithelial layer and the layer of cones cut at various levels. Acetic bichromate material, stained heavily with picro-indigo-carmine. × 560.

PLATE 28.

[All the figures are from drawings of preparations of the retina of Sphenodon punctatus. They have been outlined in every case by camera lucida.]

Fig. 4.—Portion of a vertical section, half way between the macula lutea and ora serrata, to give a general view of the arrangement of the various layers of the retina. The preparation was from material fixed with Fleming's solution, stained in bulk with borax carmine and on the slide with brazilin. × 440.

Fig. 5.—Vertical section through the layer of cones, showing nine cones, six of which are ordinary single ones, three double. Of two of the latter, however, only the near cone is in focus. The preparation is from material preserved in Zenker's fluid, stained in bulk with borax carmine and on the slide with picro-nigrosine. × 1120.

Fig. 6.—Two ordinary single cones to show the structure of the outer segments. The outer segment of (*a*) is broken up into transverse discs, while that of (*b*) shows the spiral twisting sometimes seen in the inner half of the outer segment. The preparation is from material preserved in Flemming, and stained with an attempt at the Weigert Pal method (see "Methods," p. 308). × 1120.

Fig. 7.—One ordinary single and one double cone to show their general structure and the arrangement of their nuclei and fibrils in the outer nuclear layer. The outer segments have been broken away in the course of preparation. Staining as for fig. 6. × 1120.

Fig. 8.—Portion of layer of cone-cells to show position and structure of two small single cones, (*c*) and (*d*), and their nuclei in relation to the ordinary single and double cones. No outer segments are shown. Staining as for fig. 6. × 1120.

Fig. 9.—Tangential section of portion of the cone-layer to show the arrangement of double and single cones and their structural detail. (*e*), (*f*), (*g*), (*h*) and (*j*) are various double cones referred to in the text. Preparation as for fig. 2. × 1120.

Fig. 10.—Tangential section through the outer nuclear layer at the level of the nuclei of the cone-cells. From an eye fixed in acetic bichromate and stained on the slide with Ehrlich's hæmatoxylin. × 1120.

PLATE 29.

[All the figures are from drawings or preparations of the retina of Sphenodon punctatus, and they have been outlined by camera lucida.]

Fig. 11.—Vertical section of portions of the inner molecular and inner nuclear layers. The three kinds of cells which are present in the inner nuclear layer are distinguishable by the size and shape of their nuclei. Staining as for fig. 6. × 1120.

Fig. 12.—Portion of vertical section of the nerve-fibre, ganglion-cell and inner molecular layers, to show the arrangement of the nerve-fibres in bundles and their position and that of the ganglion-cells. The figure also shows the trumpet-shaped bases of Müller's fibres which support the retina. Staining as for fig. 6. × 560.

Fig. 13.—Vertical section of the retina at the ora serrata, showing the transition between the various layers of the retina proper and the columnar epithelial cells of the pars ciliaris retinæ. The pigment layer had been pulled away in the preparation and has been partly restored. Staining as for fig. 6. × 560.

Fig. 14.—Tangential section through the outer portions of several pigment epithelial cells, showing their hexagonal and pentagonal shapes, and the arrangement of the protoplasm in blocks. Staining as for fig. 3. × 1120.